In the Specification:

In the specification, please make the following amendments:

In ¶ [0022]

[0022] The current invention is directed to α-MSH-related peptides. More specifically, the alpha-MSH ~~related~~ peptides have been structurally modified from alpha-MSH. These modified alpha MSH peptides are contemplated for use in antimicrobial therapy to treat infections. The modified α-MSH peptides maintain advantages over other antimicrobial therapy in that they are less likely to generate resistant microbial strains and are virtually non-toxic to mammalian cells. Infections can include those of bacterial, viral, parasitic and fungal origin.

In ¶ [0023]

[0023] The α-isoform of melanocyte-stimulating hormone (MSH) (SEQ. ID NO. 13) is a naturally occurring 13-amino acid peptide. α-MSH (SEQ. ID NO. 13) and its carboxy-terminal tripeptide, Lys-Pro-Val (SEQ. ID NO. 16), each have potent anti-inflammatory properties and have exhibited antimicrobial properties toward two representative classes of organisms, fungus and bacteria: *S. Aureus* and *Candida Albicans*, respectively. Catania, A, et al., *"Antimicrobial Effects of α-MSH Peptides,"* Journal of Leukocyte Biology 67: 233-239, 2000; see also, Catania, A., et al., *"Anti-Inflammatory Influence of the Immunomodulator α-MSH."* Immunology Today 18: 140-45, 1997. The α-, β-, and γ-MSH peptides are derived from post-translation processing and of the precursor protein pro-opiomelanocortin. Pro-opiomelanocortin is expressed

in the pituitary gland, in two brain nuclei, and in several peripheral tissues. Effects of melanocortins have been described on behavior, metabolism, fever, inflammation, analgesia, addiction, nerve regeneration, and the cardiovascular system. The presence of the ancient anti-inflammatory peptide α-melanocyte-stimulating hormone [α-MSH (1-13), SYSMEHFRWGKPV] (SEQ. ID NO. 13) in barrier organs, such as gut and skin, suggests a role in nonspecific, or innate, host defense.

In ¶ [0024]

[0024] α-MSH peptides significantly inhibit *S. aureus* colony formation and reverse the enhancing effect of urokinase on colony formation. α-MSH (SEQ. ID NO. 13) antimicrobial effects occur over a broad range of concentrations, including the physiological (picomolar) range. Small concentrations of α-MSH peptides likewise reduce viability and germ tube formation of the yeast *C. albicans*.

In ¶ [0025]

[0025] Antimicrobial influences of α-MSH peptides could be mediated by their capacity to increase cellular cAMP. cAMP is significantly augmented in peptide-treated yeast. Reduced killing of pathogens is a detrimental consequence of therapy with anti-inflammatory drugs. Because α-MSH (SEQ. ID NO. 13) has potent anti-inflammatory effects, its influence on *C. albicans* and *S. aureus* killing by human neutrophils has been determined. α-MSH peptides do not reduce killing, but rather enhance it, likely as a consequence of the direct antimicrobial activity. α-MSH peptides that combine antipyretic, anti-inflammatory, and antimicrobial effects may be useful in the treatment of disorders in which infection and inflammation coexist.

In ¶ [0027]

[0027] In one preferred embodiment of the invention, a peptide is prepared that comprises R1-Lys-X1-Val (SEQ. ID. NO. 1), where Val is the carboxy-terminal amino acid and X1 is either Phe or DPhe and where R1 is His-Phe-Arg-Trp-Gly. In another preferred embodiment of the invention, a peptide is prepared that contains His-X2-Arg-Trp-Gly-Lys-Pro-Val (SEQ. ID. NO. 2), where X2 is, D-Phe, or DNleal. This can be combined with SEQ. ID NO. 1 via a Gly-Lys bond giving His-X2-Arg-Trp-Gly-Lsy-X1-Val (SEQ. ID NO. 3.) Here, the sequences are connected through a Gly-Lys peptide bond resulting in a peptide where Val is the carboxy-terminal amino acid.

In ¶ [0032]

[0032] In another preferred embodiment of the invention, a peptide is prepared consisting of R1-Lys-X7-Val (SEQ. ID. NO. 8) where Val is the carboxy-terminal amino acid and where X7 is an amino acid having a negatively charged functional group. Negatively charged functional group amino acids may be selected from the group consisting of Asp, Glu, and their D-isomers thereof. In another preferred embodiment of the invention, a peptide is prepared where SEQ. ID NO. 2, His-X2-Arg-Trp-Gly is connected to the Lys of SEQ. ID NO. 8, giving His-X2-Arg-Trp-Gly-Lys-X7-Val (SEQ. ID. NO. 9), and where X2, as above, is DPhe or DNleal. Similar to above, SEQ. ID NO. 2 and SEQ. ID NO. 7 are connected via a Gly-Lys.. In other words, SEQ. ID NO. 2 replaces the R1 in SEQ. ID NO. 7.

In ¶ [0037]

[0037] The references cited above and below are incorporated by reference as if fully set forth herein. The current invention is directed to novel modified α-MSH-~~related~~ peptides that have use in antimicrobial therapy. The invention maintains advantages over other antimicrobial therapy in that it is less likely to generate resistant microbial strains, maintains balance between strains of bacteria while helping to combat infection and it is virtually non-toxic to mammalian cells. Bacterial, parasitic, viral and fungal infections are contemplated.

In ¶ [0039]

[0039] Unmodified α-MSH (SEQ. ID NO. 13) is an ancient, thirteen amino-acid peptide produced by post-translational processing of the larger precursor molecule propiomelanocortin. It shares the same 1-13 amino acid sequence with adrenocorticotropic hormone ("ACTH") (SEQ. ID NO. 14), also derived from propiomelanocortin. α-MSH (SEQ. ID NO. 13) is secreted by many cell types, including pituitary cells, monocytes, melanocytes, and keratinocytes. It can be found in the skin of rats, in the human epidermis, or in the mucosal barrier of the gastrointestinal tract in intact and hypophysectomized rats. See e.g. Eberle, A. N., *"The Melanotrophins,"* Karger, Basel, Switzerland (1998); Lipton, J.M., et. al., *"Anti-inflammatory Influence of the Neuroimmunomodulator α-MSH,"* Immunol. Today 18, 140-145 (1997); Thody, A.J., et.al., *"MSH Peptides are Present in Mammalian Skin,"* Peptides 4, 813-815 (1983); Fox, J. A., et.al., *"Immunoreactive α-Melanocyte Stimulating Hormone, Its Distribution in the Gastrointestinal Tract of Intact and Hypophysectomized Rats,"* Life. Sci. 18, 2127-2132 (1981).

In ¶ [0040]

[0040] α-MSH (SEQ. ID NO. 13) and its derivatives are known to have potent antipyretic and anti-inflammatory properties, yet they have extremely low toxicity. They can reduce production of host cells' pro-inflammatory mediators *in vitro*, and can also reduce production of local and systemic reactions in animal models for inflammation. The "core" α-MSH sequence Met-Glu-His-Phe-Arg-Trp-Gly (SEQ. ID NO. 15), for example, has learning and memory behavioral effects but little antipyretic and anti-inflammatory activity. In contrast, the active message sequence for the antipyretic and anti-inflammatory activities resides in the carboxy-terminal amino-acid lys-pro-val sequence (SEQ. ID NO. 16) of α-MSH. This tripeptide has activities *in vitro* and *in vivo* that parallel but are more potent than those of the parent molecule. The anti-inflammatory activity of α-MSH (SEQ. ID NO. 13) and/or its derivatives are disclosed in the following two patents which are hereby incorporated by reference: U.S. Patent No. 5,028,592, issued on July 2, 1991 to Lipton, J.M., entitled "ANTIPYRETIC AND ANTI-INFLAMMATORY LYS PRO VAL COMPOSITIONS AND METHOD OF USE;" U.S. Patent No. 5,157,023, issued on October 20, 1992 to Lipton, J.M., entitled "ANTIPREYTIC AND ANTI-INFLAMMATORY LYS PRO VAL COMPOSITIONS AND METHOD OF USE;" see also Catania, A., et. al., *"α-Melanocyte Stimulating Hormone in the Modulation of Host Reactions,"* Endocr. Rev. 14, 564-576 (1993); Lipton, J. M., et.al., *"Anti-inflammatory Influence of the Neuroimmunomodulator of α-MSH, Immunol."* Today 18, 140-145 (1997); Rajora, N,, et. al., *"α-MSH Production Receptors and Influence on Neopterin, in a Human Monocyte/macrophage Cell"* Line, J. Leukoc. Biol.

59, 248-253 (1996); Star, R.A., et. al., *"Evidence of Autocrine Modulation of Macrophage Nitric Oxide Synthase by α-MSH,"* Proc. Nat'l. Acad. Sci. (USA) 92, 8016-8020 (1995); Lipton, J.M., et. al., *"Anti-inflammatory Effects of the Neuropeptide α-MSH in Acute Chronic and Systemic inflammation,"* Ann. N.Y. Acad. Sci. 741, 137-148 (1994); Rajora, N., et.al., *"α-MSH Modulates Local and Circulating tumor Necrosis Factor α in Experimental Brain Inflammation,"* J. Neuroosci, 17, 2181-2186 (1997); Richards, D. B., et. al., *"Effect of a-MSH (11-13) (lysine-proline-valine) on Fever in the Rabbit,"* Peptides 5, 815-817 (1984); Hiltz, M. E., et. al., *"Anti-inflammatory Activity of a COOH-terminal Fragment of the Neuropeptide α-MSH,"* FASEB J. 3, 2282-2284 (1989).

In ¶ [0041]

[0041] In addition to its anti-inflammatory and anti-pyretic function, a preferred aspect of the present invention involves the anti-microbial or anti-infection activity of the modified α-MSH-related peptides and/or their derivatives. As described below, modified α-MSH ~~related~~ peptides have significant anti-infection uses.

In ¶ [0042]

[0042] Infections are not confined to a single cause. Multiple organisms and infectious agents, including bacteria, fungi, viruses and parasites, individually or in combination, can cause infection. For treatment of these infections, the novel α-MSH-~~related~~ peptides may be applied locally to the site of the infection and/or inflammation by methods known in the art. For example, modified α-MSH-~~related~~ peptides and their derivatives may be dissolved in solutions such as phosphate buffer saline, hyalurinate, methylcellulose, carboxymethlcellulose, or ethanol. Solvated α-MSH peptides may then

be combined with vehicles such as injectable solutions, tables, capsules, topical ointments, creams, gels, aerosol sprays, suppositories, liquid solutions and absorbent materials.

In ¶ [0055]

[0055] At $1x10^6$/ml in HBSS, these fungi were incubated in the presence or absence of modified α-MSH ~~-related~~ peptides at concentrations ranging from 10^{-15} to 10^{-4} M for two hours at 37°C. Cells were then washed in cold distilled water and diluted with HBSS to a concentration of 100 organisms/ml. One-milliliter aliquots were then dispensed on blood agar plates and incubated for 48 hours at 37°C. The organism's viability may be estimated from the number of colonies formed.

In ¶ [0056]

[0056] Figure 1 shows that modified α-MSH ~~-related~~ peptides greatly reduced the ability of *C. albicans* to form colonies. This demonstrates that modified α-MSH ~~-related~~ peptides can inhibit the growth of *Candida albicans*, an agent known to cause candidiasis, vaginitis, urethritis, balanoposthitis, and gastrointestinal infection in cancer patients. Bast, R., et al., "Cancer Medicine," BC Decker, Inc., p. 157-163, 2000.

In ¶ [0057]

[0057] The modified α-MSH ~~-related~~ peptides not only retain their effectiveness, they, unexpectedly, are more potent inhibitors of *C. albicans* growth relative to naturally occurring α-MSH (SEQ. ID NO. 13), which inhibited less than 80% of the colonies. See U.S. Patent Application No. 09/535,066. Applicants have designed and evaluated the antimicrobial activity of modified α-MSH peptides toward *C.*

albicans. These peptides were designed to determine the effect of sequence Lys-Pro-Val (SEQ. NO. 16) on biological activity. The minimally active His-Phe-Arg-Trp sequence (SEQ. NO. 16) was chosen. This sequence is important in interacting with melanocortin receptors, while the Lys-Pro-Val (SEQ. NO. 15) sequence is known to be important for antimicrobial activity. In an attempt to elucidate the contributions of each of the amino acids of the Lys-Pro-Val (SEQ. NO. 16) sequence toward antimicrobial activity, an alanine scan was performed. As shown in Table 1, the alanine substitutions displayed that Lys and Pro are more important than Val in activity. In contrast, replacing Val with DVal, and with Leu, did not substantially alter antimicrobial activity, showing that this residue is not crucial. The importance of Val in the Lys-Pro-Val (SEQ. NO. 16) sequence is therefore unclear, and it could be a remnant of pro-opiomelanocortin biosynthesis.

In ¶ [0058]

[0058] Replacing the Phe residue within the His-Phe-Arg sequence with DNleal resulted in increased activity in almost all peptides tested, confirming a behavior found previously in melanocortin peptides.

In ¶ [0059]

[0059] No substantial modification in activity was shown by SEQ. ID NO. 30 and SEQ. ID NO. 32~~Peptide 14 and 15~~, where Ser replaced the Pro residue in the Lys-Pro-Val sequence. Replacing the Pro residue from the Lys-Pro-Val sequence with either Asp (SEQ. ID NO. 42) or Glu (SEQ. ID NO. 44), resulted in decreasesd activity.

This result confirms earlier studies demonstrating that a negative charge in the carboxy-terminal region is deleterious for antimicrobial activity of melanocortin peptides.

In ¶ [0060]

[0060] Peptides bearing a replacement of the Pro residue from the Lys-Pro-Val sequence with either Phe (SEQ. ID NO. 37) or DPhe (SEQ. ID NO. 38), exhibited potent antimicrobial activity toward *Candida albicans*.

In ¶ [0061]

[0062] For example, SEQ. ID NO. 37~~peptide 20~~, which contains enhanced hydrophobicity but unaltered net charge, showed remarkable anticandidacidal activity suggesting that its mechanism of action is different from that of other antimicrobial peptides. Most other antimicrobial peptides alter membrane permeability and impair internal homeostasis of the organism. No evidence suggests that α-MSH (SEQ. ID NO. 13) and its analogues operate in this way. Because the overall positive charge of α-MSH peptides is very low relative to other antimicrobial peptides, it appears the positive charge alone does not account for the antimicrobial activity.

In ¶ [0063]

[0063] Recent reports indicate that the candidacidal effect of α-MSH is mediated through cAMP induction. Catania, A., et al., *"Antimicrobial Effects of α-MSH Peptides,"* Journal of Leukocyte Biology 67: 233-239, 2000. It is likely, therefore, that the modified α-MSH-related peptides enhance intracellular cAMP levels and thereby induced toxicity.

EXAMPLE II: GENERATION OF A MODIFIED α-MSH PEPTIDE

[0064] This example illustrates the generation of a novel peptide by modifying an α-MSH peptide (SEQ. ID NO. 13). SEQ. ID NO. 37~~Peptide No. 20~~ is chosen here for this example. This is a representative example of how all of the peptide sequences in Table 1 were created. By adding the desired amino acids during synthesis of the growing peptide chain, each of the peptide sequences can be generated. All peptides were synthesized by solid-phase peptide synthesis followed by RP-HPLC purification.

In ¶ [0065]

[0065] The peptides were synthesized on 0.15 g of Wang resin (substitution 0.7 mmol/g) by manual methods using N^{α}-Fmoc chemistry and an orthogonal side chain protection strategy. The entire synthesis was performed under an argon atmosphere. The resin was swollen in DCM / DMF (1:1) for 2 hours. To generate ~~peptide No. 20~~SEQ. ID NO. 37, the following amino acids were added by stepwise addition: Fmoc-His(N^{im}-Trt)-OH, N^{α}-Fmoc-DNle~~a~~l-OH, N^{α}-Fmoc-Arg(N^{g}-Pbf)-OH, N^{α}-Fmoc-Trp-OH, N^{α}-Fmoc-Gly-OH, N^{α}-Fmoc-Lys-OH, N^{α}-Fmoc-Phe-OH, N^{α}-Fmoc-Val-OH, using standard solid phase methods. Each coupling reaction was achieved using a 3-fold excess each of the amino acids, HBTU, and HOBt in the presence of a 6-fold excess of DIPEA for 1 h. De-protection of the N^{α}-Fmoc group was carried out by treating the protected peptide resin with 25% piperidine solution in DMF (1 x 4 mL, 5 min., 1 x 4 mL, 20 min). After each coupling and de-protection, the peptide resin was washed with DMF (3 x 4 mL), DCM (3 x 50 mL) and then again with DMF. The peptide sequences were thus assembled by alternate cycles of coupling and de-

protection. After coupling of the carboxy-terminal amino acid, the amino-terminal Fmoc group was de-blocked as before and after the peptide-resin was thoroughly washed with DCM (4 x 25 mL) and dried under an argon atmosphere to yield dried peptide-resin.

In ¶ [0067]

[0067] Final peptide purification was achieved using a preparative RP-HPLC Vydac C18 (218TP1520, 15μm). The peptides were injected onto the column at a concentration of 20-30 mg/mL in 20% aqueous CH_3CN. They were eluted with a CH_3CN gradient (0 to 55%) over 35 minutes at a flow rate of 15.0 mL/min, with a constant concentration of TFA (0.1% v/v). The separations were monitored at 230 nm and 280 nm and integrated with a Shimadzu diode array detector (SPD-M10A VP dual wavelength absorbency detector model UV-D). Fractions corresponding to the major peak were collected, pooled, and lyophilized to yield the final peptides as pure (>95%) white solids. Amino acid analyses were carried out using a Pico-Tag Work Station. Lyophilized samples of peptides (50-1000 pmol) were hydrolyzed in heat-treated borosilicate tubes (4x50 mm) using the Pico-Tag Work Station (Waters-Millipore, Waltham, MA) for 1hour at 150 °C with 200 ml 6 N HCl containing 1% phenol; a Pico-Tag column (3.9x15 mm) was employed to separate the amino acid derivatives. The analytical data for each compound is presented in Table 1.

Please amend Table 1 as follows:

Table 1

~~No.~~ SEQ. ID NO.	~~Code~~	Structure	% Inhib.	SD
~~1~~18	~~MSH-3~~	His-Phe-Arg-Trp-Gly-Lys-Pro-*Dval*	82.5	26.8
~~2~~19	~~MSH-4~~	His-Phe-Arg-Trp-Gly-*Ala*-Pro-Val	26.2	29.1
~~3~~20	~~MSH-5~~	His-Phe-Arg-Trp-Gly-Lys-*Ala*-Val	12.8	18.1
~~4~~21	~~MSH-6~~	His-Phe-Arg-Trp-Gly-Lys-Pro-*Ala*	68.4	31.5
~~5~~22	~~MSH-7~~	His-*DPhe*-Arg-Trp-Gly-Lys-Pro-Val	79.4	27.3
~~6~~23	~~MSH-8~~	His-*DNleal*-Arg-Trp-Gly-Lys-Pro-Val	95.3	7.7
~~7~~24	~~MSH-9~~	His-Phe-Arg-*DTrp*-Gly-Lys-Pro-Val	81.9	24.5
~~8~~25	~~MSH-10~~	His-Phe-Arg-Trp-Gly-Lys-Pro-*Leu*	86.6	23.2
~~9~~26	~~MSH-12~~	His-Phe-Arg-Trp-Gly-Lys-*DAla*-Val	43.7	29.5
~~10~~27	~~MSH-17~~	His-DNleal-Arg-Trp-Gly-Lys-*Ala*-Val	28.0	24.5
~~11~~28	~~MSH-18~~	His-DNleal-Arg-Trp-Gly-Lys-*DAla*-Val	69.2	27.1
~~12~~29	~~MSH-13~~	His-Phe-Arg-Trp-Gly-Lys-*Gly*-Val	41.8	27.9
~~13~~30	~~MSH-19~~	His-DNleal-Arg-Trp-Gly-Lys-*Gly*-Val	36.2	24.2
~~14~~31	~~MSH-14~~	His-Phe-Arg-Trp-Gly-Lys-*Ser*-Val	32.3	25.5
~~15~~32	~~MSH-20~~	His-DNleal-Arg-Trp-Gly-Lys-*Ser*-Val	73.9	25.0
~~16~~33	~~MSH-15~~	His-Phe-Arg-Trp-Gly-Lys-*Phe*-Val	90.0	9.3
~~17~~34	~~MSH-27~~	His-Phe-Arg-Trp-Gly-Lys-*DPhe*-Val	97.5	4.2
~~18~~35	~~MSH-28~~	His-DPhe-Arg-Trp-Gly-Lys-*Phe*-Val	89.6	14.5
~~19~~36	~~MSH-30~~	His-DPhe-Arg-Trp-Gly-Lys-*DPhe*-Val	82.0	24.9
~~20~~37	~~MSH-21~~	His-DNleal-Arg-Trp-Gly-Lys-*Phe*-Val	99.7	0.6
~~21~~38	~~MSH-31~~	His-DNleal-Arg-Trp-Gly-Lys-*DPhe*-Val	57.6	26.7
~~22~~39	~~MSH-16~~	His-Phe-Arg-Trp-Gly-Lys-*Asp*-Val	5.9	9.1
~~23~~40	~~MSH-32~~	His-Phe-Arg-Trp-Gly-Lys-*DAsp*-Val	15.7	15.6
~~24~~41	~~MSH-29~~	His-DPhe-Arg-Trp-Gly-Lys-*Asp*-Val	3.7	4.9
~~25~~42	~~MSH-22~~	His-DNleal-Arg-Trp-Gly-Lys-*Asp*-Val	16.8	22.3
~~26~~43	~~MSH-23~~	His-Phe-Arg-Trp-Gly-Lys-*Glu*-Val	11.2	12.7
~~27~~44	~~MSH-24~~	His-DNleal-Arg-Trp-Gly-Lys-*Glu*-Val	32.3	25.6
~~28~~45	~~MSH-25~~	His-Phe-Arg-Trp-Gly-Lys-*Lys*-Val	41.0	26.9
~~29~~46	~~MSH-26~~	His-DNleal-Arg-Trp-Gly-Lys-*Lys*-Val	85.4	26.1